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Wound dressings impregnated with cuprous oxide microparticles have broad spectrum antimicrobial efficacy

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Abstract:

Copper is naturally antibacterial. The FDA and other regulatory organizations have approved the use of wound dressings impregnated with cuprous oxide microparticles (hereinafter referred to as COD). Microbiological viability of a variety of microorganisms was decreased by more than 10,000fold (4 logs) after just three hours of exposure to COD at 37 °C (p 0.001). Efficacy of COD dressings that have been naturally aged for three years were shown to have the same microbial titer decreases. Even after seven consecutive daily inoculations of the dressings with 106 CFU, the COD's significant biocidal effectiveness was maintained. Even after 7 daily inoculations of various bacteria on the outside surface of the dressings, COD with an adhesive contour stopped the flow of germs from the outside environment to the wound bed side of the dressing. The research shows that the cuprous oxide impregnated dressings have broad range powerful in vitro biocidal effectiveness against a wide array of microorganisms when used as a whole.

Keywords:The anti-microbial and biocidal properties of copper

Introduction

Wounds, notably those infected by bacteria, are becoming more common due to the aging and expanding global population [1]. As a result of wound infections, wound healing may be severely delayed because of multiple processes that inhibit the wound's inflammatory phase from transitioning into the wound's proliferative phase, which results in wound chronicity. Because of the rise in the number of bacteria that are resistant to antibiotics, treating many wound infections successfully has become more challenging [3]. Antimicrobial therapy's effectiveness may be reduced or even eliminated if a microscopicbiofilm forms on the wound. A microbial infection complicates one surgical procedure out of every twenty-four [4]. A considerable number of people and health care systems are affected by wound infections [5,6]. As a clinical practice in wound treatment, wound dressings play a significant role in protecting wounds from microorganisms and preventing and treating wound infections [3]. Microorganisms need copper as a micronutrient to thrive. Many bacteria contain copper chaperons and particular copper pumps to manage the intracellular concentration of copper because copper ions may generate reactive oxygen species (ROS). A microorganism's ability to deal with excess copper is compromised when it is subjected to a concentration of cop per that is too high. Damage to microorganisms is caused by a variety of nonspecific mechanisms, including permeabilization of the plasma membrane, membrane lipid peroxidation, damage to nucleic acids, and inhibition of intracellular protein construction and function [11]. As a result of the multisite, nonspecific processes that cause copper damage, it is very difficult for bacteria to become copper-tolerant [11,12]. Dressings that include antimicrobial agents are becoming more commonplace for treating and preventing infection of wounds. [13] In the past, copper oxide microparticle-based wound dressings were documented for their development, safety, and biocidal qualities [14]. Wound dressings containing cuprous oxide microparticles that have been certified for use by many regulatory authorities and have lately come into use for the treatment of acute and chronic wounds are described in this article.

Methods and supplies

Dresses to Try Out:

The production of cuprous oxide-impregnated prototype wound dressings and the impregnation of microparticles of cuprous oxide in polymeric materials were previously described [14,18]. An absorbent layer is sandwiched between one or two non-binding hydrophilic nonwoven polypropylene layers in cuprous oxide-impregnated dressings (CODs). Cuprous oxide microparticles are infused throughout each layer (Figure 1). The wound exudates may be absorbed by the highly absorbent orange layer that is put directly on the wound bed. The wound dressings are able to absorb ten times their own weight in water. ' Wound cavities and deep wounds are more suited to CODs with two exterior layers. COD dressings that do not have an adhesive contour may be trimmed to the wound's shape and size. The COD with an adhesive shape is more suited for wounds that have recently undergone surgery.

SEM (Jeol JSM-IT100, Japan) and X-ray photoelectron spectrum analysis (built-in system of JEOL) proved the presence of copper in the COD, as shown in Figure 1.

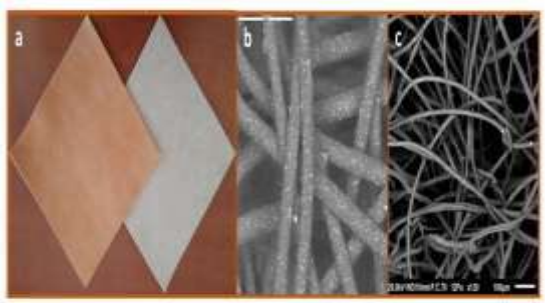


Figure:1

Wound dressings infused with cuprous oxide. The COD is made up of a layer that is extremely absorbent (beige) and one or two non-adherent exterior layers (orange) (a). Microparticles of cuprous oxide are seen as white spots in the scanning electronic microscopy pictures of the orange layer (b) and the absorbent layer (a) (c). Similarly constructed but copper-free wound dressings (e.g., 3M Life Sterile Dressings; Hubei QianjiangKingphur Medical Materials Co Ltd., Hubei, China) have been utilized as negative controls. It was also compared to the following commercially available antimicrobial wound dressings containing silver: Maxorb extra AG (Medline Industries, Northfield, IL; Puracol Plus (Medline Industries; Northfield; Optifoam (Medline Industries; Northfield; Calcium Alginate (McKesson Corporation; Irving, TX; Tegaderm Alginate Ag (3M, Saint Paul, MN; Biatain Alginate Ag (Coloplast, Fredensborg, Denmark), Acticoat Flex 3 (S; Acticoat Flex 3; S; Optifoam; Medline Industries; Northfield; Calcium Alginate (McKesson Corporation)

Reduction in Viable Microbial Titers by Logs

Test Method 100-1993 of the AATCC was shown to be compatible with COD's antibacterial and antifungal properties. In the absence of specific instructions, we followed the following procedure: Six 3.3 cm by 3.3 cm square swatches were cut for both the test and control wound dressings. Sterilized plastic bottles held the samples at the bottom. With the addition of two mL of a 0.85 percent saline/0.1 percent Tween 80 (ST; Sigma Aldrich Israel Ltd., Rehovot, Israel) solution containing wound exudate surrogate, all liquid was completely absorbed into the control and test samples (Biological Industries, Beit-Haemek, Israel). Afterward, 100 L of microbiological stock assay solution was applied to each control and test swatch sample, making sure that all the liquid was absorbed. Within minutes after receiving the "Time 0" samples, the lab administered a 100-mL neutralizing solution solution to each one.

The other containers containing the test samples were sealed and put in a 37 °C incubator for either 1, 3, or 18 h. (Candida albicans). 100 mL of D/E was then added to the samples. Sterilized Stomager bags were used to store the D/E neutralized swatches immediately (Time 0) or at the end of incubation (Alex Red Ltd. Mevasseret Zion, Israel). They were ingested and returned to their containers at this period. Using 0.45 m Cellulose Nitrate Filters (SartoriousStedim Biotech GmbH, Göttingen, Germany), each vessel's recovered liquids were filtered using Pall filtering equipment (Pall Corporation, Port Washington, NY, USA). After washing the filters twice with 100 mL of ST, the membranes were then put on petri plates with CHROMagarTM Orientation agar (http://www.chro- magar.com) and incubated at either 24 °C or 37 °C to determine the presence of microorganisms, respectively. After 24–48 hours of incubation, CFU were counted. The detection limit was lowered from 100 CFU to 10 CFU by filtering 10 mL of each liquid in numerous experiments. The percentage of bacterial or fungal reduction was calculated using the following formula: It was necessary to divide the number of challenge organisms recovered from infected test samples by the number of test organisms recovered from infected test samples using this formula: Percent R = 100(A B)/A There are a number of bacteria in this study that have shown resistant to the antibiotic methicillin (ATCC BAA-1708), as well as other common pathogens (ATCC 10231). At least two experiments were carried out, with at least three replicates per sample. The mean and SD for each experiment are shown.

Results

Figure 2 depicts the substantial drop in viable titer of all microorganisms exposed to the COD, with the notable exception of Candida albicans, which achieved the 10,000-fold reduction in 18 h of incubation. The titers of all microbes exposed to the negative control dressings at 37 °C, on the other hand, did not change or even grew with time (negative log decrease). In a sample trial depicted in Figure 3, there was no difference in the biocidal efficiency of dressings aged 7 months, 15 months, or 3 years. Using 106 CFU of Klebsiella pneumoniae

every day for seven days, researchers tested the COD's ability to reduce bacterial titers by more than 10,000 times when exposed to high bacterial titerseveryday. Incubation at 37°C for 24 hours after the seventh bacterial injection was followed by the removal of bacteria from the COD and control dressing swatches and the determination of CFU. All four COD samples had no live bacteria, suggesting an over 10,000-fold decrease of viable bacteria titer compared to the control samples (Fig. 4). In addition, we tested the antibacterial efficiency of the COD against a variety of silver-containing antimicrobial wound dressings that are currently available. To test viability, the bacteria were collected after 1 h of incubation at 37 degrees Celsius. Even while no living bacteria were found after one hour of incubation with the COD, Mepilex Ag, and Acticoat Ag, the biocidal efficiency of the other dressings evaluated was much lower (Ta ble
1).

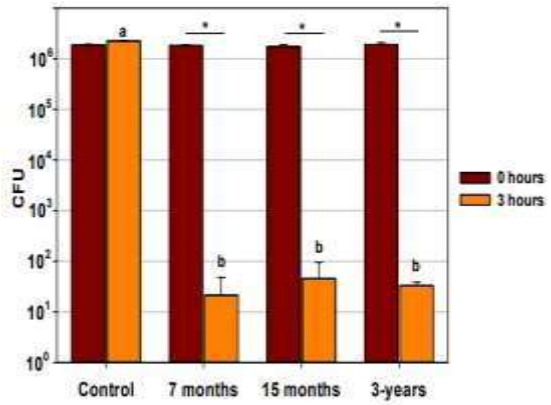


Figure 3. The biocidal efficacy of organically matured, three-year-old COD. Seven months, fifteen months, and three years of naturally aged COD were injected with Enterobacter aerogenes. Every COD tested showed a drop of over 10,000-fold after three hours of incubation. Analyzing statistical differences in COD titers after three hours of incubation between the negative control and each naturally aged COD was done using the Holm-Sidak procedure and One Way Analysis of Variance. This study employed t-tests to look for significant differences between the microbiological titers after 3 hours of incubation and the beginning titer for each naturally aged COD (0 hours). Results obtained from 3 hours of incubation on Control Dressings (a) and 3 hours of incubation on COD (b) show statistical significance (p 0.001).

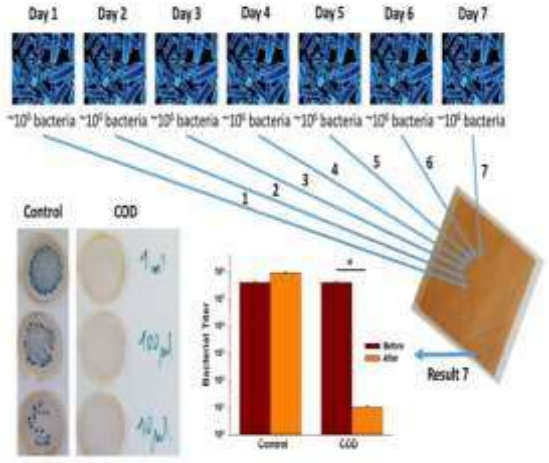
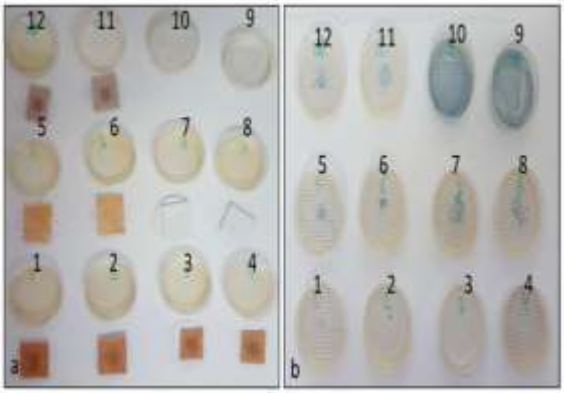


Figure 4. Multiple bacterial exposures to test dressings resulted in significant biocidal effectiveness. 106 CFU of Klebsiella pneumoniae were added to each of the eight dressings, four of which served as controls and four of which served as tests, seven days a week for seven days. A 24hour incubation at 37 °C followed the first six daily bacterial inoculations of the swatches. Filtration of 10 L, 100 L, and 1 mL of the stomached microbial solution recovered the bacteria after the 7th bacterial injection on Day 7 at 37°C. After 24 hours of incubation at 37 °C, the CFUs of the surviving bacteria were determined. The CFU collected from a control swatch of clothing and a COD swatch is illustrated in This is an illustration of what I mean. Bacterial titres increased dramatically on Day 7, with recovered titers well beyond the expected range.in a bar graph presented By using a t-test, we compared the CFU titers of the spiking bacteria (before) with those of the recovered bacteria (after). To put it another way, * = 0.001Using the methods outlined in Methods section and shown in Figure 5, we tested whether different COD structures without an adhesive contour might restrict bacteria's migration from the outside environment to the wound bed by adding 1 mL of Klebsiella pneumoniae CFUs to the test swatches. A comparable number of bacteria were found to pass through the COD internal layer alone, the COD exterior layer alone, the control internal layer alone, and the control internal layer alone without the inclusion of cuprous oxide microparticles (Figure 5). However, only one or three live bacteria, and no viable bacteria, traveled from one side of the fabric to the other via the two-layer COD and the threelayer COD, respectively. Even after 7 days of inoculating the dressings with a variety of gramnegative and gram-positive bacteria on their outer surfaces, the COD with an adhesive contour was still able to prevent bacteria from passing into the wound bed, as shown in Figure 6.

There was no further bacterial growth on the agar plates following the last bacterial injection and the removal of the wound dressings from the agar. Under all of the control wound dressings, however, bacterial growth could be shown on the agar plate (Figure 6)



#	Test Item	CFU
1;2	2-layer COD	3;1
3,4	3-layer COD	0;0
5,6	COD External layer + Control Internal layer	50;45
7,8	COD internal layer only	42;60
9,10	No Dressing	TMTC* ; TMTC
11,12	Control Internal layer only	70;80

Figure 5. Without an adhesive contour, bacterial passage through the COD is reduced. Cultivated Klebsiella pneumoniae was inserted into each swatch in the center of the test items as shown in the left-hand column of the Figure. (a) (dark spot seen in each swatch). Removed test items were put on top of agar plates after 10 minutes, after which the filters were placed on top. Two agar plates were inoculated with 1 mL of the Klebsiella pneumoniae stock solution as a control of the bacterial burden (plates 9 and 10). CFU on each plate were counted after an overnight incubation period at 37 °C. In the right column of the Figure, you can see the number of CFUs found in each of the examined duplicate samples. TMTC: There are just too many to list.

Discussion

Acute and chronic wounds may now be treated using wound dressings coated with cuprous oxide microparticles, thanks to approvals from the FDA in the United States (510(k) K180643), the EU, and other regulatory organizations across the globe. For more than two years, these wound dressings have been in clinical use in numerous countries, increasing wound healing in both infected and noninfected wounds, including chronic wounds [15– 17]. The results of our research show that COD have a good biocidal action against a wide range of bacteria on the dressings and protect the wounds from external microbial contamination. As a result of their remarkable wide-spectrum biocidal characteristics (e.g., [20]), cuprous oxide microparticles were selected as the active biocidal component in the COD dressings to be utilized. In order to keep the wound dressings free of biocontamination and to reduce the entry of viable microorganisms from the surrounding environment into the wound bed, cuprous oxide microparticles act as a reservoir for copper ions that are slowly and continuously released [16,21]. Both gram-positive and gram-negative bacteria, as well as MRSA, an antibiotic-resistant bacterium, were successfully treated with the dressings. For our antimicrobial assays, we employ bacteria that have been shown to be wound pathogens [22,23]. These bacteria create blue colonies on CHROMagarTM Orientation agar that are simple to count for CFU counts in several of the studies (Figures 3–5). Consequently, we used them as representative microorganisms in some of the experiments. Using an initial inoculum titer of 106 CFU and a detection limit of 102 CFU, we were able to show at least 10,000-fold decreases in the microbial titer of the dressings. Indeed, bacterial titer decreases of tens of thousands of fold were seen within three hours of contact to the dressings. To show that the dressings had long-lasting powerful biocidal action even after 7 consecutive days of daily spikings of COD to high bacterial titers, a 10,000fold decrease was obtained after the 7th bacterial inoculation. That's why we inoculated the dressings every 7 days, since each COD has been approved by regulatory organizations to be used for a maximum of 7 consecutive days. To obtain a 10,000-fold decrease in Candida albicans, it was necessary to use a longer exposure duration. In general, bacteria are more susceptible to copper exposure than yeast and fungus [24]. To be clear, the lowest detection limit of most assays was typically under 100 CFU (2logs). Consequently, the number of bacteria recovered when no CFUs were identified was taken as 100 CFUs. The largest fold decrease recorded in these instances was 10,000 since the original inoculum was 106 CFU. A far greater decrease in 10 fold may have been achieved, as was the case in certain experiments (data not shown). Silver, which has antibacterial characteristics of its own, is now used in the majority of antimicrobial dressings used in clinical

settings. Silver dressings, however, are increasingly being linked to reports of wound healing inhibition and toxicity [25–29]. Since the COD showed significantly better antimicrobial efficacy in our hands than most commercially available antimicrobial silver wound dressings that we tested (Table 1), this means that in clinical settings, its potential to provide antimicrobial protection is on par with or even greater than the vast majority of currently used silver dressings (and thus, probably superior). It is made up of two main layers. the equivalent of ten times its own weight in absorption capacity (data not shown). As a result, wound exudates, the majority of which come from infected wounds, may be more easily absorbed by the body. The ability to absorb and retain wound exudates is an important wound-dressing attribute because wound exudate management is critical to wound healing [30,31]. As a result of the cuprous oxide microparticles in this layer, it is possible to reduce the danger of cross-contamination and the smell of a wound infection. Afterwards, the dressing may be easily removed from the wound bed thanks to the nonwoven fabric's thin, nonstick layer, which enables wound exudate to travel to the absorbent layer. To further strengthen their ability to block the entry of live bacteria into the wound bed, this layer also includes cuprous oxide microparticles (Figure 5). To prevent wound dressings from sticking to the surrounding wound bed, a thin nonwoven layer is provided on both sides of the absorbent fabric in one of the COD constructions. This construction is used to manage wound cavities and wound tunnels. A mechanical barrier against microbiological contamination is also provided by the COD with an adhesive coating (Figure 6). The reported COD have much superior biocidal characteristics and better exudate management qualities than the prototypes previously published [14]. The COD with an adhesive contour additionally functions as a mechanical barrier to microbial contamination. It is shown that cuprous oxide microparticle-coated wound dressings have a long-lasting biocidal and wound microbial protection efficiency as well as the ability to treat infected wounds with COD.

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